

THE LOCALIZATION OF A MASON-PFIZER MONKEY VIRUS-RELATED ANTIGEN IN JAAGSIEKTE TUMOUR TISSUE AND CELL LINES

ANNA-LISE PAYNE⁽¹⁾, D. W. VERWOERD⁽¹⁾ and HELEN M. GARNETT⁽²⁾

ABSTRACT

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Mason-Pfizer monkey virus-related antigen was detected in 3 out of 5 jaagsiekte lungs examined using a direct immunoperoxidase staining technique with anti-MPMV p27 serum. Most of the antigen was localized in the alveolar lumina of the lesions. The reaction was further characterised on immune blots and found to involve a protein with a molecular mass of 29 000 daltons (JSRV p29). JSRV p29 antigen was also detected in 2 jaagsiekte cell lines.

INTRODUCTION

Jaagsiekte (ovine pulmonary adenomatosis) is an infectious lung cancer in sheep caused by jaagsiekte retrovirus (JSRV) (Verwoerd, Williamson & De Villiers, 1980; Verwoerd & Williamson, 1982). Electron microscopic (EM) studies of tumour tissue from natural cases (Wandera & Krauss, 1971; Perk & Hod, 1971; Bucciarelli, 1973; Hod, Herz & Zimmer, 1977) as well as experimentally induced cases of jaagsiekte (Payne, Verwoerd & Garnett, 1983) indicate that virus particles are relatively rare. JSRV was observed in the alveolar lumina as well as associated with the tumour cells. However, it is possible that there is more viral antigen present in jaagsiekte lesions than complete virions. Sharp & Herring (1983) reported a serological cross-reaction between jaagsiekte lung material, of Scottish origin, and both Mason-Pfizer monkey virus (MPMV) and mouse mammary tumour virus (MMTV). These results complement the EM results (Payne *et al.*, 1983) as well as the biochemical results (Verwoerd, Williamson & De Villiers, 1980; Verwoerd, Payne, York & Myer, 1983) which indicate that JSRV has morphological and biochemical similarities to both groups of viruses. Therefore, anti-MPMV serum was used for the immunocytochemical localization of JSRV antigen in infected lungs and cell lines and to ascertain the JSRV protein responsible for this cross reactivity.

MATERIALS AND METHODS

Source of antisera and conjugates

Two different batches of goat anti-Mason-Pfizer monkey virus p27 were obtained from the National Cancer Institute (NCI), Bethesda, USA. In a radioimmunoassay test against MPMV p27, the first batch of serum (lot number = 74S000148) had a titre of 46 700 and the second batch of serum (lot number = 74S000157) had a titre of 38 800. Goat anti-lentivirus serum was obtained from a goat infected intratracheally with the SA-OMVV-1 strain of lentivirus (Payne, York, Verwoerd, De Villiers, Quérat, Barban, Sauze & Vigne, 1986). Normal sheep serum was obtained from a clinically normal sheep. Goat anti-rabbit immunoglobulin peroxidase conjugate was obtained from Bio-Rad, USA. Rabbit anti-sheep immunoglobulin peroxidase conjugate was obtained from Dakopatts, Copenhagen, Denmark. All antisera were titrated and the maximum dilution giving optimum results for the particular technique was used in each experiment.

⁽¹⁾ Molecular Biology Section, Onderstepoort 0110

⁽²⁾ Department of Microbiology, University of the Witwatersrand, Johannesburg 2000

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Lung tissue

Lung tissue for frozen sections was obtained from 8 animals. The history and pathology of each animal is outlined in Table 1. The samples were generally taken from lobes that had been tied off to prevent them from being washed out with medium during the process of virus collection for other experiments. Each sample was cut into a 3 mm cube, snap frozen in an isopentane slush, wrapped individually in aluminium foil, placed in plastic vials (Nunc) and stored in liquid nitrogen. Frozen sections were cut on a Reichert Jung Cryo-Cut II microtome. The sections were air dried, covered in parafilm and stored at -20 °C until staining.

TABLE 1 Summary of the pathology of the ovine lungs used for frozen section immunocytochemistry

Sheep No	Age at PM	Pathology*
6675	4 months	Jaagsiekte with macrophage infiltration. Mild interstitial thickening
5169/1	2 months	Jaagsiekte with macrophage infiltration and slight lymphoid hyperplasia
4976	2 months	Jaagsiekte with purulent and interstitial pneumonia
4887	3 weeks	Jaagsiekte with exudative pneumonia
5160	2 weeks	Jaagsiekte negative although injected with JSRV. Exudative bronchopneumonia with interstitial thickening
5135	5 weeks	Jaagsiekte with macrophages and neutrophils
6226	1 week	Normal lung
B sheep	Adult	Normal lung

* All jaagsiekte animals had been experimentally infected with JSRV. (PM = post-mortem)

Cell lines and growth conditions

Two epithelial cell lines from jaagsiekte lung tissue (15.4 and 21.3 cells) (Coetzee, Els & Verwoerd, 1976) were cultivated in F12 medium with 10 % foetal calf serum, penicillin, streptomycin and nystatin. Ovine foetal tracheal cells (OFTR cells) were kindly provided by Dr H. D. Lehmkuhl, National Animal Disease Centre, Iowa. The OFTR cells were cultivated using Dulbecco's MEM with 10 % foetal calf serum as well as penicillin and streptomycin. All the cell cultures were cultivated on 2-chamber slides (Miles Laboratories).

Preparation of peroxidase conjugate

An anti-MPMV peroxidase conjugate was used to stain frozen sections. Immunoglobulins were precipitated from anti-MPMV serum by adding an equal volume of saturated ammonium sulphate solution. The precipitate was dialysed overnight in 0.1 M phosphate buffer, pH 6.8. The conjugate was prepared according to Avrameas (1969).

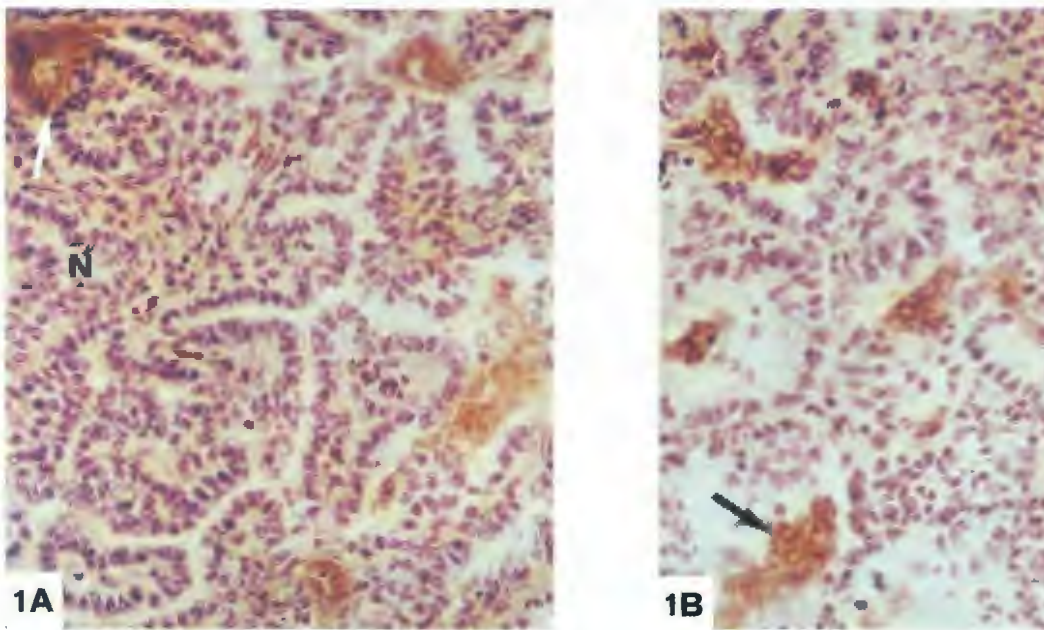


FIG. 1 MPMV-related antigen detected by a peroxidase conjugate in a frozen section of a jaagsiekte lung. The brown stain represents the antigen and the blue stain the counter-stained nuclei. 1A—Antigen was detected in close association with the apical region of tumour cells (arrow). Note the negative alveolus (N) adjacent to the positive one. $\times 200$. 1B—Antigen was mostly located in the alveolar lumina (arrow) of the lesions. $\times 200$

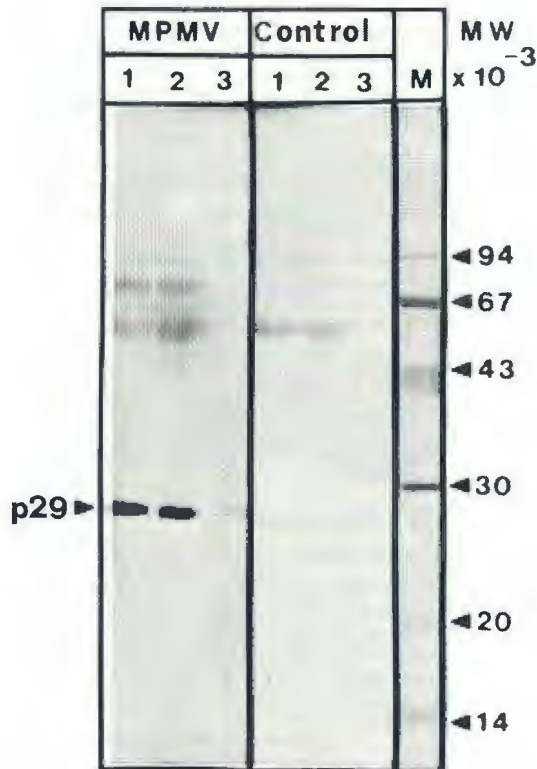


FIG. 2 An immunoblot assay on electrophoretically separated polypeptides in untreated lung rinse pellet (1), freon extracted lung rinse pellet (2) and freon extracted lung rinse pellet followed by gel filtration on a Sephacryl 1000 column (3). The left hand side has been reacted with anti-MPMV serum (MPMV) followed by anti-sheep immunoglobulin peroxidase conjugate and the right hand side with the conjugate and no primary antiserum (control). M indicates low molecular mass markers stained with amido black

Immunoperoxidase method for immunocytochemistry

A direct staining method was used to detect MPMV-related antigens in lung sections to avoid cross-reactions with the immunoglobulins in the sections. An indirect staining technique was used to detect MPMV-related antigen in cell lines. Frozen sections were fixed in 96 % ethanol for a few minutes and then transferred to phosphate buffered saline (PBS). Cultured cells were washed well with PBS, air dried, fixed for 2 minutes in 96 % ethanol, air dried and then transferred to PBS. In both sections and cultured cells, non-specific protein binding sites were blocked with 3 % bovine serum albumin (BSA) for 10 minutes before incubation with the primary antibody (indirect method) or the anti-MPMV peroxidase conjugate (direct method) for 2 hours. All serum and conjugate dilutions were made in PBS containing 3 % BSA. Anti-MPMV serum was diluted 1:50 to stain cell lines, anti-MPMV batch 1 peroxidase conjugate was diluted 1:100 and the anti-MPMV batch 2 peroxidase conjugate was diluted 1:20. Controls included normal sheep serum as well as rabbit anti-sheep immunoglobulin peroxidase conjugate on cultured cells and goat anti-rabbit immunoglobulin peroxidase conjugate on frozen sections. The sections were then washed with 0,5 % Tween 20 in PBS followed by 2 further washes in PBS. In the indirect method anti-sheep immunoglobulin peroxidase conjugate was diluted 1:200 in 3 % BSA in PBS and applied to the cells for 1 hour. The slides were washed as above and then incubated for 10 minutes in a freshly prepared substrate solution [10 mg 3'3 diaminobenzidine tetrahydrochloride/grade II/(Sigma) in 20 ml PBS containing 0,01 % H₂O₂]. The sections were then washed under running tap water, stained with haematoxylin for 2 minutes, blued under running tap water, dehydrated in an ethanol series and mounted in Permount.

Immunoblot assay

Partially purified JSRV (Verwoerd *et al.*, 1983) or MPMV (supplied by NCI, Bethesda) as well as pellets and supernatant fractions from jaagsiekte (JS) lung-rinse material were fractionated electrophoretically in 12,5 %

polyacrylamide gel containing 0,1 % SDS with a 4 % stacking gel according to the method of King & Laemmli (1971). The viral samples were dissociated by heating in a glycerol bath (90 °C) for 3 minutes in 2,5 % SDS, 5 % mercaptoethanol and 12,5 % glycerol. The amount of sample in each well varied according to the protein concentration of the sample. The low molecular mass calibration kit supplied by Pharmacia Fine Chemicals AB, Uppsala, Sweden was used to provide molecular mass markers on each gel run. After electrophoresis Serva blue dye was introduced into each well to mark the lanes. This facilitated identifying the lanes on the nitrocellulose membrane after blotting, therefore making it easier to cut the membrane into strips.

The proteins were transferred to nitrocellulose (Schleicher and Schuell, 0,45 μ m pore size, BA85) in a Bio-Rad transblot apparatus at a current of 0,45 Amps for 4,5 hours at 4 °C.

The nitrocellulose membrane was removed from the Bio-Rad blotting apparatus and cut into strips. The low molecular mass markers were stained with amido black (0,1 g amido black, 10 ml acetic acid, 45 ml distilled water) for 3 minutes and then destained in amido black destaining solution (450 ml methanol, 40 ml water, 10 ml acetic acid) for 10 min. The strips to be stained with antibody were blocked with 50 % horse serum in PBS for 1 hour. The immobilized polypeptides were then reacted overnight with goat anti-MPMV p27 serum (or anti-lentivirus serum), diluted 1:50 in 50 % horse serum in PBS. This was followed by 3 \times 5 min washes in PBS containing 0,5 M NaCl, 0,001 M EDTA and 0,5 % Tween 20 (Sharp & Herring, 1983). The nitrocellulose strips were then incubated for a further hour with rabbit anti-sheep immunoglobulin peroxidase conjugate diluted 1:400 in 50 % horse serum. After washing, the strips were transferred through 2 changes of distilled water and then into the substrate [60 mg 4-chloro-1-naphthol (Bio-Rad, USA) dissolved in 20 ml ice cold methanol added to 100 ml PBS containing 60 μ l 30 % H₂O₂]. When a colour reaction was observed the membrane strips were transferred to distilled water and then dried.

RESULTS

MPMV-related antigen was detected in 3 of the 5 jaagsiekte lungs examined (Table 2) using immunocytochemical techniques on frozen lung sections. However, these results were only demonstrated with the peroxidase conjugate made from the first batch of anti-MPMV serum and not with the second batch. The peroxidase conjugate made from the second batch of serum consistently gave negative results on all frozen sections despite the fact that the conjugate gave good results on the immunoblot assay against MPMV. Therefore, the following results were obtained using conjugates made from the first batch of anti-MPMV p27 serum. It was necessary to use a direct staining technique utilizing an anti-MPMV peroxidase conjugate, to detect antigen in frozen sections, because the anti-MPMV serum was produced in a goat and anti-goat immunoglobulin conjugates cross-react with normal immunoglobulins in the sheep lung sections. Frozen sections were used to preserve the antigenicity of the protein. The antigen was always found in close association with recognizable tumour cells in the alveolar lumen and in some cases the apical section of the tumour cells appeared to be stained (Fig. 1A). Most of the antigen appeared to be localized in alveolar lumina (Fig. 1B). Not all lesions were producing detectable antigen and it was common to find a highly positive alveolar lumen adjacent to a completely negative one. There did not appear to be any staining of the interstitial layers.

The bronchiolar lumina were usually completely negative for MPMV-related antigen. However, in some sections there was staining of the smooth muscle around the bronchioles. This staining was considered to be non-specific as it was also observed in negative lungs. The goat anti-rabbit conjugate gave negative results. No endogenous peroxidase activity was observed in frozen lung sections in the areas that were positive with the anti-MPMV peroxidase conjugate. However, some endogenous peroxidase activity was observed in the blood-vessels of some lung sections.

TABLE 2 Results of staining of frozen sections with anti-MPMV peroxidase conjugate

Sheep No	Disease	Result
6675	JS	+ve
5161/9	JS	low +ve
4976	JS & PN	+ve
4887	JS & PN	-ve
5135	JS	-ve
5160	PN	-ve
6226	NSL	-ve
B sheep	NSL	-ve

(JS—jaagsiekte; PN—pneumonia; NSL—normal sheep lung)

Utilizing an immunoblot assay, the nature of the antigen detected in the frozen sections was characterized. The molecular mass of the MPMV-related protein was estimated to be 29 000 daltons (p29). This protein was detected at various stages of the JSRV purification including crude pellets obtained from JS lung washes, freon extracted pellets and column purified material (Fig. 2). The p29 band was rather faint in the column purified material but this is due to a dilution effect rather than removal of the protein, the pellet being diluted during the purification procedure. The protein could not be demonstrated in the supernatant fraction of the lung wash material. These results indicated that the JSRV p29 was associated with particulate fractions of the lung washes and not in the form of free protein. However, it should be noted that there was a large amount of immunoglobulin present in the supernatant fraction of lung washes which resulted in overloading of the gels. Hence small quantities of JSRV p29 may not be detected readily in the supernatants without further purification. An anti-sheep immunoglobulin peroxidase conjugate control was included in the immunoblot assay to identify any background immunoglobulin bands. This was necessary because the viral pellets from lung washes have been previously shown to contain immunoglobulins (Verwoerd *et al.*, 1983) and in an indirect assay these bands would be detected by the conjugate. No bands were detected in the JSRV p29 region by the conjugate but when used alone there was a band in the 60 000 dalton region which probably represented the immunoglobulin fraction. The anti-lentivirus control was also negative indicating that the lung wash pellet was not contaminated with lentivirus antigen. The second batch of anti-MPMV serum gave very weak positive results on the immunoblot assay.

Utilizing an indirect immunoperoxidase technique (with the first batch of anti-MPMV serum) it was found that MPMV-related antigen was sporadically produced in both the 15.4 and the 21.3 cell lines when they were in the logarithmic phase of growth (Fig. 3). The antigen was always found in the cytoplasm and the 21.3 cell line appeared to have more antigen producing cells than the 15.4 cell line. In some cells the entire cytoplasm was stained but it was more common to see staining around the periphery of the cell (Fig. 4). Sometimes there was a very concentrated spot of staining which resembled a

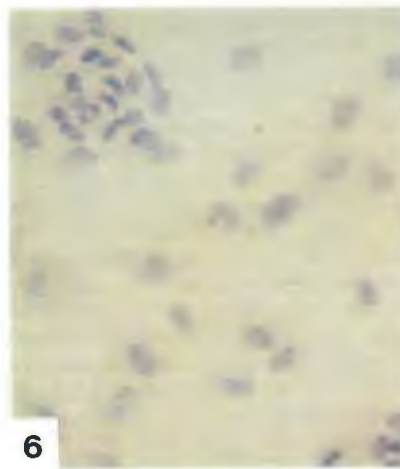
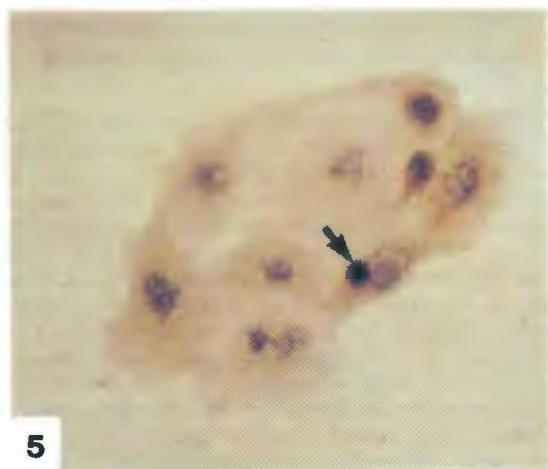
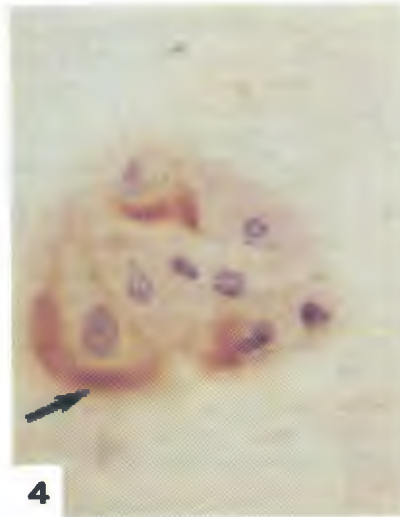
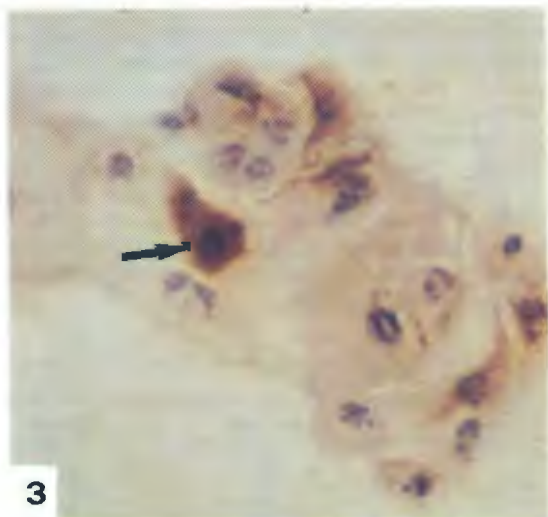


FIG. 3 MPMV-related antigen in 15.4 cells which was detected using an indirect immunoperoxidase technique. Note the highly positive cell (arrow) adjacent to negative cells. $\times 200$.

FIG. 4 MPMV-related antigen on the periphery of the cytoplasm (arrow) of a 15.4 cell. $\times 200$.

FIG. 5 MPMV-related antigen in an "inclusion body" in the cytoplasm (arrow) of a 21.3 cell. $\times 200$.

FIG. 6 OFTR cells were negative for MPMV-related antigen as detected by the indirect immunoperoxidase technique. $\times 200$.

cytoplasmic inclusion body (Fig. 5). The producing cells were often adjacent to completely negative cells. Once the cells were confluent no antigen production could be detected. No antigen production was detected in the control OFTR cells (Fig. 6). Negative sheep serum gave no staining in any of the cells examined. No endogenous peroxidase activity was detected.

DISCUSSION

The serological cross-reaction between jaagsiekte retrovirus protein and anti-MPMV p27 serum, that was reported by Sharp & Herring (1983), was confirmed by an immunoblot assay. However, the first batch of anti-MPMV serum gave much better results than the second batch of antiserum. This may have been due to the difference in titre between the 2 antisera or a deterioration in the second batch of antiserum after titration. The first batch of antiserum had a higher titre against MPMV p27 and was more specific than the second batch of antiserum (NCI, datasheet). This suggests that the relationship between the 2 viruses is distal, as good positive results were only obtained when a serum with an extremely high titre was utilized in the indirect peroxidase immunoblot

assay. It is relevant to note that Sharp & Herring (1983) utilized radiolabelling techniques, which are more sensitive than enzyme labelling techniques, to detect the positive reaction on their blots. A similar antigen has been detected in lung fluid pellets from South Africa, Scotland, Peru and Israel (Sharp, personal communication), which implies that the jaagsiekte retrovirus is serologically related, if not identical, in these countries. In the current study, virus pelleted from lung rinse material from jaagsiekte infected sheep produced a prominent JSRV p29 band in an immunoblot assay using the first batch of anti-MPMV serum. However, as the virus was not completely purified it could be argued that a tumour-associated protein was bound to the virus particles. Attempts to demonstrate free antigen in the supernatants of lung wash and lung fluid pellets failed, suggesting that JSRV p29 is virus associated. However, a protein of identical molecular mass appears to be a minor protein band in polypeptide patterns of virus purified from lung-rinse pellets (Verwoerd *et al.*, 1983).

The ability of the anti-MPMV serum to detect a jaagsiekte related antigen in South African sheep made an immunocytochemical procedure to localize jaagsiekte

antigen in sheep lungs a feasible proposition. The MPMV-related antigen (JSRV p29) was detected in 3 out of 5 jaagsiekte lesions examined. Only the peroxidase conjugate made from the first batch of anti-MPMV p27 serum detected antigen in the lung. This was probably due to the lower titre of the second batch of antiserum. The location of the antigen in the lung sections correlated with the location of whole virus particles visualized by EM. However, antigen was detected in 60 % of the lungs examined using these immunocytochemical methods compared to 22 % of the lungs using EM techniques (Payne *et al.*, 1983). JSRV p29 antigen was not produced by all tumour lesions and was located in the alveolar lumen adjacent to tumour lesions as well as the apical region of tumour cells. No JSRV p29 antigen was detected in the bronchioles. However, because of the small number of samples examined, it cannot be said that the antigen is never produced in this region.

The MPMV-related antigen was also detected in both jaagsiekte tumour cell lines examined. The production of the antigen by the cells was transient, being detected only in sub-confluent, actively growing cultures. Both these cell lines can induce jaagsiekte by transplantation (Verwoerd, De Villiers & Tustin, 1980). Subcellular fractions from 15.4 cells of the correct density for retrovirus can also induce jaagsiekte (Verwoerd, Williamson & De Villiers, 1980) indicating that JSRV is produced by these cell lines in low quantities. Reverse transcriptase activity and JSRV-like particles had also been detected in 15.4 cell cultures but not the 21.3 cell cultures (unpublished data). Therefore, it is not surprising that viral antigen production was detected in these cells.

The fact that only a small percentage of jaagsiekte tumour cells produced viral antigen *in vivo* or *in vitro* indicates that the cells within the tumour vary in their expression of JSRV p29 antigen. Heterogeneity in antigen expression has been reported in a number of tumour systems including mouse mammary tumours (Hager & Heppner, 1982). MMTV associated antigens are expressed differentially in mammary tumours of different murine species, among mammary tumours of the same species (Colcher, Horan Hand, Teramota, Wunderlich & Schlom, 1981) and in different areas of the same mammary tumour (Hager & Heppner, 1982). In the current study MPMV-related antigen was only produced in 3 out of 5 jaagsiekte sheep lungs examined and not all the cells in any one lesion were producing antigen. In the mouse mammary tumour system expression of viral antigens does not correlate with tumorigenicity (Hager & Heppner, 1982). A similar situation may exist in jaagsiekte where, although antigen production appears to be sporadic in the tumour cells, the cells retain their tumorigenicity. The viral antigen production in tumour cells may be related to the stage in the cell cycle or the physiological state of the cell. Type B oncovirus production appears to be dependent upon the secretory activity of the tumour whereas Type C oncovirus synthesis is dependent on mitosis (Schopper, Fasske, Fetting & Thermann, 1983). JSRV can be isolated from the lung washes of animals with jaagsiekte, induced by transplantation of tumour cells. This suggests that the cells may produce more JSRV *in vivo* than *in vitro* due to an absence of the correct growth factors to stimulate virus production *in vitro*. It is important to know if the difference in antigen production in cell cultures is due to the heterogeneity of the cell population or the physiological state of the cells as this may affect *in vitro* production of the virus, which has been a problem to date.

In conclusion, the results of the present study demonstrate that JSRV p29, which is related to MPMV p27, is present in tumour cells *in vivo* and *in vitro*. This should allow for the development of a specific assay system for JSRV detection as well as assisting in the development of better techniques for *in vitro* virus production.

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REFERENCES

- AVRAMEAS, S., 1969. Coupling of enzymes to proteins with glutaraldehyde. Use of conjugates for the detection of antigen and antibodies. *Immunocytochemistry*, 6, 43-52.
- BUCCIARELLI, E., 1973. L'adenomatosi pulmonare della pecora (jaagsiekte) studio ultrastrutturale. *Lav Ana Pat Perugia*, 33, 99-117.
- COETZEE, S., ELS, H. J. & VERWOERD, D. W., 1976. Transmission of jaagsiekte (ovine pulmonary adenomatosis) by means of a permanent cell line established from affected lungs. *Onderstepoort Journal of Veterinary Research*, 43, 133-142.
- COLCHER, D., HORAN HAND, P., TERAMOTA, Y. A., WUNDERLICH, D. & SCHLOM, J., 1981. Use of monoclonal antibodies to define the diversity of mammary tumor viral gene products in virions and mammary tumors of the genus *Mus*. *Cancer Research*, 41, 1451-1459.
- HAGER, J. C. & HEPPNER, G. H., 1982. Heterogeneity of expression and induction of mouse mammary tumour virus antigens in mouse mammary tumours. *Cancer Research*, 42, 4325-4329.
- HOD, I., HERZ, A. & ZIMMER, A., 1977. Pulmonary carcinoma (jaagsiekte) of sheep. Ultrastructural study of early and advanced tumour lesions. *American Journal of Pathology*, 86, 545-558.
- KING, J. & LAEMMLI, U.K., 1971. Polypeptides of the tail fibres of bacteriophage T4. *Journal of Molecular Biology*, 62, 465-473.
- PAYNE, A., VERWOERD, D. W. & GARNETT, H. M., 1983. The morphology and morphogenesis of jaagsiekte retrovirus (JSRV). *Onderstepoort Journal of Veterinary Research*, 50, 317-322.
- PAYNE, A., YORK, D. F., VERWOERD, D. W., DE VILLIERS, E. M., QUÉRAT, G., BARBAN, V., SAUZE, N. & VIGNE, R., 1986. Isolation and identification of a South African lentivirus form jaagsiekte lungs. *Onderstepoort Journal of Veterinary Research*, 53, 55-62.
- PERK, K. & HOD, I., 1971. Demonstration of virus particles in ovine pulmonary adenomatosis. *Experientia*, 27 (4), 437-438.
- SCHOPPER, CHR., FASSKE, E., FETTING, R. & THERMANN, H., 1983. The correlation between tissue differentiation and production of mammary tumour virus (MTV) in transplanted murine mammary tumours. *Journal of Cancer Research and Clinical Oncology*, 105, 127-133.
- SHARP, J. M. & HERRING, A. J., 1983. Sheep pulmonary adenomatosis: Demonstration of a protein which cross reacts with the major core proteins of Mason-Pfizer monkey virus and mouse mammary tumour virus. *Journal of General Virology*, 64, 2323-2327.
- VERWOERD, D. W., DE VILLIERS, E. M. & TUSTIN, R. C., 1980. Aetiology of Jaagsiekte: Experimental transmission to lambs by means of cultured cells and cell homogenates. *Onderstepoort Journal of Veterinary Research*, 47, 13-18.
- VERWOERD, D. W., WILLIAMSON, A. & DE VILLIERS, E. M., 1980. Aetiology of jaagsiekte: Transmission by means of subcellular fractions and evidence for the involvement of a retrovirus. *Onderstepoort Journal of Veterinary Research*, 47, 275-280.
- VERWOERD, D. W. & WILLIAMSON, A., 1982. Preliminary characterization of a newly isolated ovine retrovirus causing jaagsiekte, a pulmonary adenomatosis. In: JOHN, D. S. & BLAKESLEE, J. S. (eds), *Advances in comparative leukemia research 1981*. Elsevier North Holland, Inc.
- VERWOERD, D. W., PAYNE, A., YORK, D. F. & MYER, M. S., 1983. Isolation and preliminary characterization of the jaagsiekte retrovirus. *Onderstepoort Journal of Veterinary Research*, 50, 309-316.
- WANDERA, J. G. & KRAUSS, H., 1971. The ultrastructure of sheep pulmonary adenomatosis. *Zentralblatt für Veterinärmedizin*, 18, 325-334.